[3] The formulas for the ζ^i_α, the A_{ij} and the following quantity $G_0(M, \alpha)$ are given in the paper "On Curved Shock Waves," *J. Math. Physics*, **26**, 62–68 (1947). A more explicit formula for G_0 is to be found in the article "Calculation of the Curvatures of Attached Shock Waves," *Ibid.*, **27**, 279–297 (1949).

[4] This relation is derived in §7 of the forthcoming article "The Determination of Pressure on Curved Bodies Behind Shocks," *Communications on Applied Mathematics, New York Univ., Inst. for Math. and Mech.*

[5] See §5 of ref. 4.

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*THE SELECTIVE ADVANTAGE OF AN ADENINELESS DOUBLE MUTANT OVER ONE OF THE SINGLE MUTANTS INVOLVED**

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In discussions of evolutionary specialization it is often suggested that if one reaction in a biosynthesis is blocked, the ability to carry out other reactions preceding this one in the series will tend to be lost as a result of mutation, provided that the intermediates involved are not themselves useful. Such a tendency would be predicted if mutations which result in loss of synthetic ability were more frequent, or of greater selective advantage than those which restore the ability. The possibility that loss of synthetic ability may sometimes confer a selective advantage provides an interpretation of the behavior of the adenineless strain of Neurospora which is discussed here. In each of three stock cultures of this strain there occurred spontaneously a second mutation to adenineless, and the cultures became genetically pure for the double mutants. Selection of the double mutants may be accounted for by the fact that, on the medium used, the double mutant in each reaches its maximum rate of growth more quickly than does the original single mutant.

Detection of the Spontaneous Mutations.—A striking characteristic of the strain in which the new mutations appeared makes detection of the presence of certain other adenineless mutant genes quite simple. The accumulation by this strain (isolation number 35203) of a purple pigment is prevented if any one of three other adenineless mutant genes (isolation numbers 27663, 28610 and 44411) is present.[1] Available evidence indicates that the purple pigment is a derivative of an intermediate in the biosynthesis of adenine and that accumulation of the pigment is prevented by the 27663, 28610 and 44411 mutations because these interfere with reactions which come earlier in the series, thereby cutting off the supply of pigment precursor.[1] Hence

the single mutant, 35203, is purple while the double mutant of 35203 with 27663, 28610 or 44411 is not purple.

The three isolates of ad-p (purple-adenineless), which were observed to have lost the purple character but to have retained the requirement for adenine, had been kept as stock cultures at room temperature and had been transferred at six- to eight-week intervals. Some time after the disappearance of the purple character they were out-crossed to wild type and segregation of two adenineless mutants, one of which produced the purple pigment, was demonstrated in all perithecia tested. The double mutant in each case was not purple.

Comparison of Growth Rates of the Single and Double Mutants.—The genetic constitutions of the three double mutants obtained from the above crosses were checked by out-crossing them to wild type. Also double

TABLE 1

STRAIN	GROWTH RATES (MM./HR.) COMPLETE MEDIUM	MINIMAL MEDIUM + ADENINE*
ad-p, isolate 1	2.9	3.5
ad-p, isolate 2	2.6	3.4
ad-p, isolate 3	2.8	3.4
ad-p, isolate 4	2.6	3.4
ad-p, isolate 5	2.8	3.5
ad-x1, ad-p	3.5	3.7
ad-x2, ad-p	3.5	3.6
ad-x3, ad-p	3.5	3.9
27663, ad-p	3.5	3.5
44411, ad-p	3.6	3.8
ad-p, isolate 1 + ad-x1, ad-p	3.5	3.6
ad-p, isolate 1 + ad-x2, ad-p	3.5	3.6
ad-p, isolate 1 + ad-x3, ad-p	3.5	3.8
ad-p, isolate 1 + 27663, ad-p	3.5	
ad-p, isolate 1 + 44411, ad-p	3.5	

* Adenine sulfate, 0.5 mg. per 10 ml. medium.

mutants of ad-p with two of the not-purple mutants, 27663 and 44411, were prepared. Growth rates were measured at 25°C. on complete medium in growth tubes.[2] Innocula were obtained from complete agar slant cultures which were from 7 to 10 days old. The tubes were marked at the mycelial frontier 15 to 20 hours after inoculation and the growth rate determined for the following 48-hour period. Results are given in table 1, where the three spontaneous mutations are designated as ad-x1, ad-x2 and ad-x3. It will be seen that on the complete medium the single mutant ad-p grows more slowly than any of the double mutants. Also, in tubes which were inoculated with a mixture of spores from ad-p and any one of the double mutants the rate of growth was the same as that of the double mutant. No purple pigment was formed in these tubes, except near the

point of inoculation. The growth rate of the purple strain is not constant during this 48-hour period, but is increasing, and after 60 to 70 hours approaches that of the other strains. However, it would appear that, in mixed cultures, the purple strain is forced out very early, since the pigment has been observed only very near the point of inoculation and the rate of growth is fairly constant 15 or 20 hours after inoculation.

The complete medium used had the same composition as that upon which the stock cultures had been kept. It consisted of the usual minimal medium[3] supplemented with an autolyzate of wild-type Neurospora mycelium, prepared as described by Lein, Mitchell and Houlahan.[4] A concentration of autolyzate equivalent to 40 mg. moist weight of mycelium per ml. of medium was used. On minimal medium supplemented with adenine there is little difference in the growth rate of ad-p and that of the other strains in the 48-hour period considered, but there is a greater difference during the first 15 hours after inoculation, the distance along the tube covered by ad-p being about two-thirds of that covered by the other strains. Apparently the difference is sufficient to allow selection of the double mutant on this medium too, since no pigment was observed in mixed cultures except near the innoculum.

In liquid culture, if the autolyzate is used as a supplement, after 30 to 40 hours growth the dry weight of mycelium from the double mutants and mixed cultures is 4 to 5 times greater than that from the purple mutant. After 50 to 60 hours the difference in dry weight is only about 16%, but no pigment was observed in the mixed cultures. When adenine was used as a supplement (1 mg. adenine sulfate per 20 ml. of medium) there was little difference in the dry weights after 40 hours, and the mixed cultures were purple.

Genetic Relationships of the Spontaneous Mutants and the Induced, Not-Purple Adenineless Mutants.—From crosses of the double mutants to wild type the three new mutants, ad-x1, -x2 and -x3 were obtained without ad-p. They were crossed with each other, with 27663, 28610 and 44411, and with a fourth not-purple adenineless mutant, 3254, which gives a faintly purple double mutant with ad-p. The crosses were made on corn meal agar slants and the ascospores obtained were suspended in sterile water and spread on agar minimal medium in petri-plates. The plates were kept at 25°C. for 12 to 14 hours after heat treatment and then examined for wild-type spores. These can be distinguished from mutant spores since the latter produce very short haphae and then stop growing on the unsupplemented medium, while growth of the wild type continues.[4]

From the results of these crosses, given in table 2, it appears that the three spontaneous mutants are all genetically different and that ad-x2 is also different from the four induced not-purple mutants. It is possible that ad-x1 is allelic with 28610 and ad-x3 with 27663; fertile crosses were not obtained in these two cases.

Discussion.—It is clear that in mixed cultures, containing ad-p and a double mutant of ad-p with one of the not-purple adenineless mutants described, the double mutant is selected on either of the two agar media used. The slower initial growth rate characteristic of ad-p appears to be adequate reason for the selection, but is possible that other less obvious factors are involved. The cause of the reduced growth rate is not known. Possibly the accumulation of pigment is in some way responsible but this has not been demonstrated. It can only be said that a mutation which prevents the accumulation of pigment removes, or compensates for, a detrimental effect of the ad-p mutation.

Fries, working with Ophiostoma, has observed three cases in which a mutant strain, after having spontaneously acquired a second mutation, has become genetically pure for the double mutant. Two of these strains were guanine-requiring and the second mutation in both was to hypozanthineless.[5] The third was an adenineless mutant in which a mutation to biotinless occurred.[6] These, of course, differ from the case reported here in that new growth requirements are introduced, but knowledge of possible interrelations in the biosyntheses concerned might diminish this difference.

TABLE 2

STRAINS	WILD-TYPE PROGENY OBSERVED	STRAINS	WILD-TYPE PROGENY OBSERVED
ad-x1 × ad-x2	++	ad-x2 × 27663	++
ad-x1 × ad-x3	++	ad-x2 × 28610	++
ad-x1 × 3254	++	ad-x2 × 44411	++
ad-x1 × 27663	++	ad-x3 × 3254	++
ad-x1 × 28610	No fertile cross	ad-x3 × 27663	No fertile cross
ad-x1 × 44411	++	ad-x3 × 28610	++
ad-x2 × ad-x3	++	ad-x3 × 44411	++
ad-x2 × 3254	++		

One may question the usefulness of speculation upon the applicability of selection of a further loss of synthetic ability as a mechanism for evolutionary specialization. The observations of Fries and those described here serve to establish that under certain laboratory conditions the phenomenon of selection does take place. Possibly it does so to a significant extent under natural circumstances as well.

It is of interest to compare the mutations which prevent the accumulation of purple pigment by ad-p with suppressors which operate on Drosophila mutants characterized by abnormally high pigment production. If the adenineless strains of Neurospora were always kept on a complex medium, and the adenine requirement not therefore recognized, then the not-purple mutations which prevent pigment production would, if they were encountered, be looked upon as suppressors of the purple character, quite analogous to suppressors of black,[7] sable[8] and perhaps others, in Drosophila. There

might be found then, at least four complete suppressors and one partial suppressor of purple, namely 27663, 28610, 44411, ad-x2 and 3254. Furthermore, there is another genetically distinct adenineless mutant, 44206, previously described,[1] but not otherwise discussed, here, which produces the purple pigment under different conditions and to a lesser extent. Hence this strain is quite different in appearance from ad-p and it might therefore be classified as an unrelated mutant suppressed by the same series of suppressors which operate on ad-p.

Summary.—In three stock cultures of an adenineless strain of Neurospora a double mutant arose as the result of spontaneous mutation to adenineless, and, in each case, the double mutant forced out the original single mutant. Under conditions which existed at the time the selection took place the double mutants show a faster initial rate of growth than the original single mutant. The three new mutations are genetically distinct and each one, in combination with the original mutant prevents accumulation of a purple pigment which is characteristic of this strain. Cultures from mixed innocula of the purple mutant with any one of the double mutants have the growth rate of the double mutant and form no pigment.

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HIGHER PROPERTIES OF PHYSICAL SYSTEMS OF CURVES

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1. A *system* S_k of ∞^3 curves in a field of force consists of curves along which a constrained motion is possible such that the pressure P is proportional to the normal component N of the force vector. Thus $P = kN$ where $k(\neq -1)$ is the constant factor of proportionality.[1]